A New Astomatous Ciliate, *Metaphrya sagittae*, gen. et sp. nov., found in the Coelom of Sagitta.

By

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During a short stay, in December of 1913, at the Misaki Marine Biological Station, Dr. YATSU kindly called my attention to a living specimen of *Sagitta*, which contained some large ciliates in the body-cavity. Upon close examination under the microscope, these were ascertained to represent a new mouthless holotrichous form of a remarkable characterization. I propose to call it *Metaphrya sagittae*, gen. & sp. nov.

Owing to the transparency of the host, much of the external characters of the parasites could be observed in their natural habitat. Fig. 1 represents the surface view of one of the four largest specimens in life. The body is pear-shaped, measuring about 0.25 *mm* by 0.13 *mm*. It is nearly transparent and quite colourless. The anterior end is distinctly narrower than the posterior, which is rather rounded. The body appears to be radially symmetrical in its structural plan excepting the fact that the anterior end is somewhat deflected towards one side. Fine but long cilia are present, not uniformly all over the body, but growing in 12 longitudinal shallow grooves of the body, so as to form as many equidistant ciliary bands. The organism is devoid of a mouth or any other external aperture; therefore, it should belong to the suborder Astomata of the order Holotricha. The cytoplasm investing the body is finely granular; its differentiation into the ectoplasm and the entoplasm is in the fresh state indistinct. Beneath this cytoplasmic layer there can be recog-

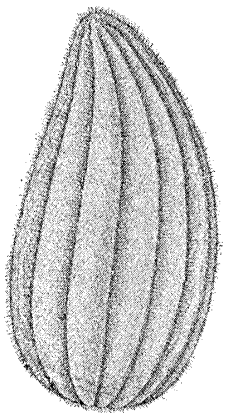

Fig. 1.
Fully grown *Metaphrya sagittae* in the living state. ×180.

nized a very thin but distinct layer, which is characterized by being strongly refractive and by extending in an irregularly wavy way when viewed in optical section. The wide space internal to this refringent layer is occupied by a clear fluid-like substance.

The parasites were fixed, together with the host, with picro-acetic acid and were in part sectioned and in part mounted in *toto*. The sections, 5μ thick, were stained with either iron-haematoxylin or DELAFIELD'S haematoxylin, with or without the use of eosin as a counter stain. Unfortunately it was found that the effects of fixing were not in all respects satisfactory. In the larger specimens the superficial cytoplasmic layer and the underlying refringent layer have become in many places widely separated from each other. It is not quite clear how such a disturbance as this was brought about only in the larger specimens. Possibly it may be due to the fixing reagent having caused contraction of the extensive refringent layer. Fig. 2 represents a median longitudinal section of a large specimen stained with DELAFIELD'S haematoxylin and eosin. The large clear spaces (marked with *) visible between the plasmic cortex and the layer of darkly stained threads representing the refringent layer that we have seen in the fresh state, are the artefacts just referred to. The same is evidently also true of the similar spaces in the central parts. One of the remarkable structural peculiarities shown by the larger, and therefore probaby full-grown, individuals consists in the fact that the cytoplasm is extraordinarily sparse in quantity as compared with the large size of the entire body. It merely forms a thin layer covering the body, the greater part of the latter being taken up by

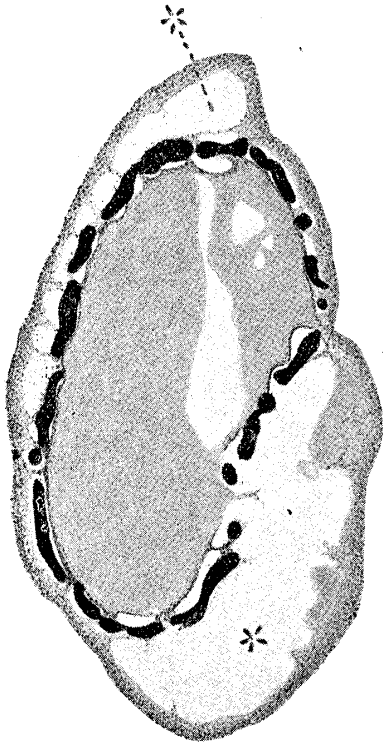

Fig. 2.
A longitudinal section of a fully grown *M. sagittae*. ×370.

the central non-plasmic substance. The cytoplasm is differentiated, though by no means sharply into the ectoplasm and the entoplasm. The former forms a very thin, uniformly finely granular superficial layer, while the latter is a little thicker and presents a reticulate granular appearance (fig. 3). The two layers show neither a structural delimitation between them nor differential staining properties.

The curious refringent layer we have seen in the fresh state, when examined in sections, presents itself as being made up of a series of deeply stained pieces arranged in a layer in the deepest parts of the entoplasm. A cursory examination of stained total preparations or of serial sections makes it at once clear that the layer in question consists in fact of comparatively thick threads which divide and anastomose, so as to bring about a network with close and irregular-shaped meshes. The substance of the threads is evidently the chromatin, and I take no heed in regarding the entire structure as the meganucleus of the organism. The entire meganucleus may thus be said to have the structure of a basketwork with a spacious hollow inside. Strikingly remarkable as is this feature of the meganucleus, it may probably be regarded to be a condition which is foreshadowed more or lass in some other astomatous ciliates, *e.g.*, *Rhizocarium concavus* CAULLERY & MESNIL *Anoplophrya alluri* CÉPÈDE, *Opalinopsis sepiolae* FOETTINGER, *etc.*

The meganuclear threads are seen in most parts to be surrounded by narrow empty spaces, which are always found to be bordered each by a distinct and dense sheet of the entoplasm. I therefore regard the spaces to be equivalent to the excretory vacuoles described by METCALF[1] from a number of *Opalina* species.

The part of the entoplasm lying internal to the meganuclear threads appears in sections as an extremely thin, very finely granular and deeply staining layer which directly invests the large central non-plasmic body. Fig. 3, which represents under a high magnification a small portion of a section belonging to the same series as that of fig. 2, illustrates all the

1) METCAL , M.M.—The excretory organs of *Opalina*, Parts I and II. Arch. f. Protist., Band X, 1907.

structures aboved described. It should further serve to illustrate an important structure not yet mentioned, *viz.*, the micronucleus. This is a small, deeply staining body which, in all the specimens examined, occurred in a single number, independently of and situated closely external to, the meganuclear basketwork. It always lies in the transverse plane passing through the middle of the body. It is of an ellipsoidal or spindle-like shape, measuring about 5μ in the major diametre. There can be distinguished in it a deeply stained peripheral and a less deeply stained central part. Around the body there always exists a narrow clear space, which does not seem to be an artificial production, since it shows itself to be stained though very lightly. There can scarcely be a doubt that the above body represents the micronucleus of the organism.

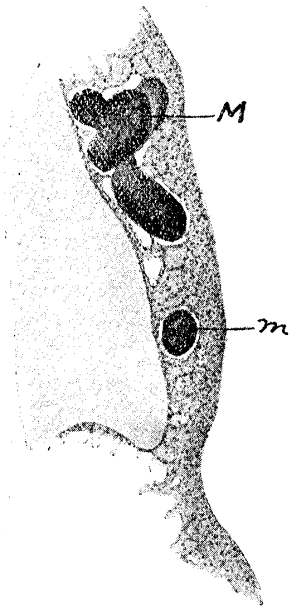

Fig. 3.

A small portion of a section through *M. sagittae*. Cytoplasmic layer to the right; central nonplasmic body to the left. M, a part of meganucleus; m, micronucleus. ×960.

The large central body differs from all the cytoplasmic structures in being perfectly homogeneous and also in being but little stainable with haematoxylin though very intensely with eosin. This peculiarity indicates that it is not protoplasmic but is probably a colloid substance of a proteidinous nature. It may be suggested that the body in question is in all probability to be looked upon as a sort of nutritive material in reserve, which is contained in, and fills up, an excessively enlarged and centrally situated vacuole.

Together with the above apparently full-grown individuals were found a number of much smaller—therefore assumably younger—specimens, which showed some important structural peculiarities. To begin with the youngest stage found, this was of an exceedingly small size in comparison mith the full-grown individuals, measuring only about 20μ by 10μ (figs. 4 and 5).

The body is covered with short cilia, which, instead of being arranged in longitudinal bands, are uniformly distributed all over. The cytoplasm presents much the same appearance as in the full-grown state. Situated in one part of the entoplasm is distinctly recognizable the micronucleus, which is of nearly the same shape and size as before described. What now form the striking features of the stage under consideration are the state of the meganucleus and the entire absence of a large central non-plasmic body. The meganucleus is not only very much smaller, but is also of a much simpler configuration, than in the fully developed state. In fact, it now consists of a relatively thick and sinuously winding thread, which may bear some short branches ending free. Some small empty-looking spaces, which I take for the excretory vacuoles, occur either close to or in direct apposition to the meganucleus. Further there occur in the entoplosm and in the vicinity of the nuclei, some small spherical, but sometimes somewhat irregular-shaped, bodies which agree in appearance and staining reactions perfectly with the substance forming the single central non-plasmic body of full-grown individuals. They may be designated the non-plasmic spherules for the sake of reference. I shall return to them presently again.

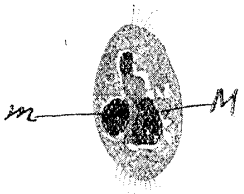

Fig. 4.
×960.

A very young individual in longitudinal seetion. ×960.

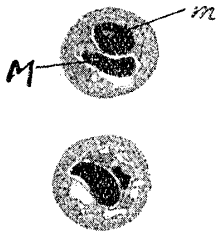

Fig. 5.

Two couse cutive cuoss-seetions through a very young indinidung. ×960.

In still larger young individuals than those described above, all the structures referred to can be more distinctly made out. The individual shown in two longitudinal sections in fig. 6 measures about 40μ by 20μ. In it, as observed on the sections, the densely granular ectoplasm can be well distinguished from the reticular entoplasm, though the two gradually merge into each other. The micronucleus is found in the usual state and can be readily recognized. The meganucleus has approached a step towards the basketwork-like structure of that of full-grown individuals, that is, it now

represents an irregular three-dimensional reticulum of the chromatin substance. Small as it still is, the meganucleus occupies a large part of the body. Its general appearance reminds one of the same organella in *Opalinopsis sepiolae* studied by DOBELL.[1] The excretory vacules are now extensively and very distinctly developed, a number of relatively large ones existing in close connection with, or directly apposed to, the meganuclear threads. In this respect, one is reminded of METCALF's illustration, especially his fig. N, of the excretory vacuoles of *Opalina*. As was stated by that author for *Opalina*, the vacuoles lack, in contrast to those described before of full-grown individuals, a definite limiting wall and seem in their nature to be nothing more than enlarged areolar spaces of the entoplasmic foam. In my opinion, the same may be said of the spaces which are occupied by the non-plasmic spherules before alluded to. In the developmental stage under consideration the same spherules are found more numerously and in a considerably larger size than in the earlier stage before descreibed, plainly indicating that they are something that grows, not only in number, but also in bulk as the organism advances in development. They lie rather closely crowded in the central parts of the body, partly inclosed in the mesh-like spaces or hollows of the yet spongy-like meganucleus (fig. 6.). I have not been able to trace subsequent changes of the non-plasmic spherules, but am of the opinion that they, at a certain stage of the development of the organism, begin to fuse together, finally to form a single mass, and that this, as the substance increases in volume, distend from within the growing meganucleus; so that, while it occupies that

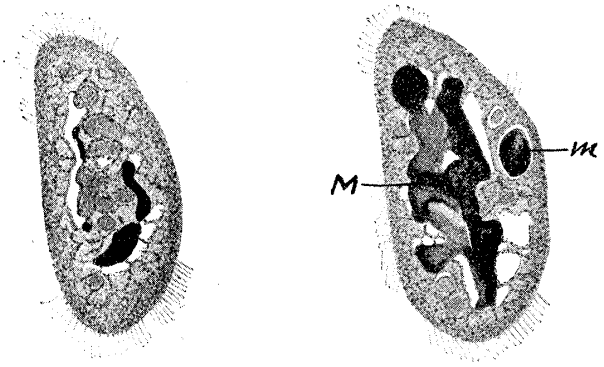

Fig. 6.
Two longitudinal sections of a young individual. ×980.

1) DOBELL, C.C.—Some observations on the Infusoria parasitic in Cephalopoda. Quart. Journ. Microscop. Sci., Vol. 53, 1909.

central position in the body, the latter assumes the form of an enveloping basketwork that we have seen in the full-grown individuals. Bodies similar to the non-plasmic spherules have been known from some astomatous infusorians. As such may be mentioned the granules observed by KOFOID[1] in *Protophrya ovicola* KOF. and interpreted by him as a highly differentiated metaplasmic substance. So likewise the peculiar spherules mentioned by METCALF[2] from both the ectoplasm and entoplasm of *Opalina*. I should think it highly probable that all the various cytoplasmic inclusions here referred to are in fact chemically nearly related substances and represent a nutritive substance stored up in the body.

Now as to the systematic position of the new genus and species. An attempt was made by CÉPÈDE[3] to exclude *Opalina*, alleged to be without micronucleus and the contractile vacuole, froms the Astomata, which group was thus made to include only the heterokaryote forms in possession of the contractile vacuole. This procedure CÉPÈDE's seems to me scarcely tenable in view of our present extended knowledge of the nature of micronucleus and of vacuoles, both contractile and non-contractile. Of the occurrence of these organellae in *Opalina*, *Opalinopsis*, and *Chromidina*, the reader is referred to the recent works of METCALF on *Opalina* and of DOBELL on *Opalinopsis sepiolae* and *Chromidina elegans*, and to the view expressed by HICKSON in LANKESTER's Treatise on Zoology (Protozoa, Part I) with regard to the presence of micronuclei in *Opalina*. The Astomata in the restricted sense was divided by CÉPÈDE into eleven families, making use of distinctive characters which, to my mind, appear to be scarcely of more than generic value. For the present at least, I should rather abide by the older scheme of LÉGER and DUBOSCQ[4] who divided the Astomata SCHEVIAKOFF simply into two

1) KOFOID, C.A.—On the structure of *Protophrya ovicola*, a ciliate infusorian from the brood-sac of *Littorina rudis* DON. The Mark Anniversary Volume, art. V, 1903.

2) METCALF M.M.—Studies on *Opalina* (preliminary notice). Zool. Anz., Bd. XXXII, 1907.

3) CÉPÈDE, C.—Recherches sur les infusoires astomes. Arch. d. Zool. Exp., tome III, 5e ser., 1910.

4) LÉGER, L., and DUBOSCQ, O.—Les Astomata representent-ils un groap naturel? Notes sur les infusoires endoparasites. Arch. Zool. Exp., 4e ser., 1904.

families, the Opalinidae and the Anoplophryiidae. The former may be defined as the Astomata which show no morphological differentiation of the chromatin into generative and vegetative nuclei (heterokaryote) and which reproduce by muttiple division or repeated transverse segmentation ; latter as those which are heterokaryote and multiply by ordinary fission.

Now then, *Metaphrya sagittae* appears to be referable to the Anoplophryiidae LÉG. & DUB. True, it somewhat approaches certain forms (*Opalinopsis*, *Chromidina*) of the Opalinidae in the peculiar form of meganucleus, and that familly generally in possessing non-contractile vacuoles instead of a single contractile vacuole ;[1] but these points I am inclind to view in the light of homoplastic convergence. The relatively complex organization of the new genus and species points to its being a highly advanced representative of the Anoplophryiidae, on which account I have chosen the generic name *Metaphrya*.

CÉPÈDE has described a goodly number of entozoic ciliates as inhabiting the body-cavities of many invertebrates. But none of them can be said to be as genuine a coelom-parasite as the present species is. *Heterophrya astomata* SIEDLECKI has often been referred to as a coelom-parasite, but the case is still open to doubt.

In conclusion, I beg to express my warmest thanks for Professor IJIMA'S kind advice and critics rendered me during my writing the present paper. I also owe very much to Dr. YATSU'S kind help in obtaining the literature.

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High Normal School, Hiroshima,
January 1916.

1) According to *Kobel'* (*l. c.*), the single vacuole of *Opalinopsis* is contractile, and the single nucleus of this form is complicately branched into a network and is never in a dispersed state as was thought formerly to be the case.